Supporting Information

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SI Methods

Biochemistry. For small-scale immunoprecipitation, S2 cells expressing HA, GFP, or Flag-tagged proteins were harvested from 15 mL of confluent culture. Cell extracts were prepared by incubating the culture with 600 μL of extraction buffer HB100 (50 mM Hepes-KOH, pH 7.6, 100 mM NaCl, 1 mM $MgCl_2$, 1 mM EGTA, 1% Triton X-100, 1 mM DTT, protease inhibitor mixture) for 20 min, followed by centrifugation at 15,000 $\times$ *g* for 15 min, and then at 100,000 $\times$ *g* for 15 min. The supernatant was added to 20 μL (bed volume) of anti-HA-conjugated agarose beads (Sigma) and rotated for 3 h, or added to 5 μL of anti-GFP or anti-Flag antibody, incubated for 2 h on ice, mixed with protein G-Sepharose beads (20 μL; Sigma) and rotated for 4 h at 4 °C. Beads were washed 4 times with HB100. Immunoprecipitation of human augmin subunits was performed in a manner identical to S2 cells, except that the starting cell culture was 20 mL.

Large-scale immunoprecipitation was performed with Dgt2-HA by using 280 mL of cell culture treated with Colcemid (3.3 μg/mL) for 18 h to accumulate mitotic cells (typically 10–20% of the cells become arrested in mitosis by this treatment). The cell extract was prepared by using HB100 and subjected to immunoprecipitation with 120 μL (bed volume) anti-HA-conjugated beads (MBL). After washing 4 times with HB100, immunoprecipitates were eluted by 0.2 M glycine (pH 2.0), followed by precipitation with trichloroacetic acid. Large-scale immunoprecipitation with GFP-hDgt6 was performed in the same way, except that the cell extracts were prepared by using HB500, which contained 500 mM NaCl, from 600 mL of cell culture treated with 100 ng/mL nocodazole for 16 h. We used 150 μL of anti-GFP-conjugated beads (MBL) for precipitation. For all experiments, untagged cells were used as the negative controls.

Rabbit polyclonal antibodies against hDgt6 were generated and affinity-purified by using mixtures of 2 synthesized peptides as antigens [PAKKSDPFQKEQDHLVE (residues 526–542) and NKSLDAKEPPSDLTR (residues 941–955)].

Immunoblotting was performed with rabbit affinity-purified anti-Dgt6 (1:50), anti-hDgt6 (1:500), mouse monoclonal anti-HA (1:200; 12CA5), anti-FLAG (1:200; M2; Sigma), and anti-GFP (1:200; Roche) antibodies. Gel filtration chromatography was carried out with Superdex 200 10/300 column attached to the AKTA system (GE Healthcare). Molecular size markers are described in ref. 1.

1. Oegema K, et al. (1999) Characterization of two related *Drosophila* gamma-tubulin complexes that differ in their ability to nucleate microtubules. *J Cell Biol* 144:721–733.

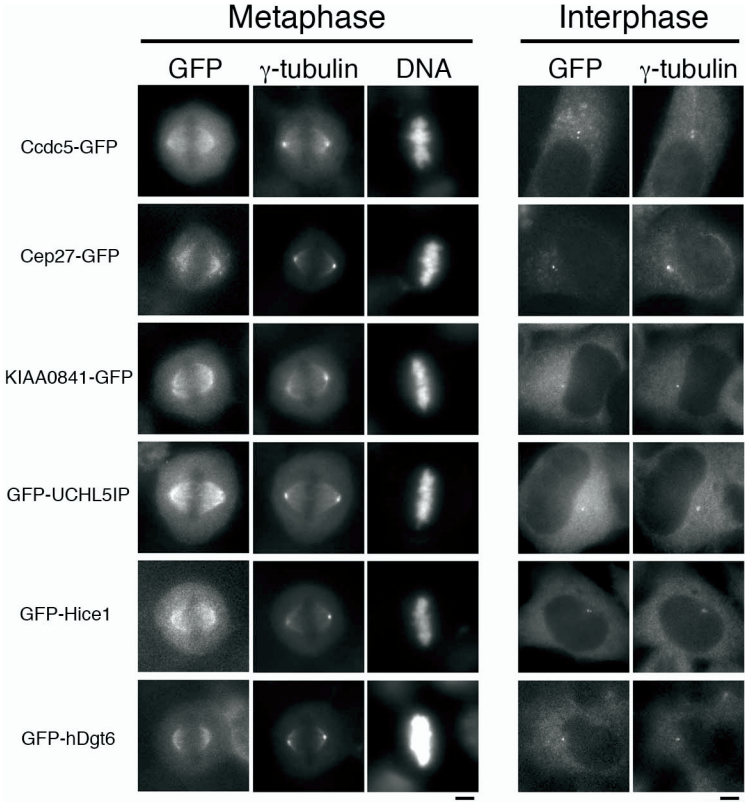


Fig. S1. Augmin localization in HeLa cells. Localization of GFP-tagged augmin subunits in metaphase and interphase is shown. The 8 proteins were abundant at the centrosome in interphase, whereas spindle localization was detected in metaphase. GFP signals were slightly more intense near the pole than around chromosomes in metaphase. Chromosomes and γ-tubulin were counterstained. (Scale bars, 5 μm.)

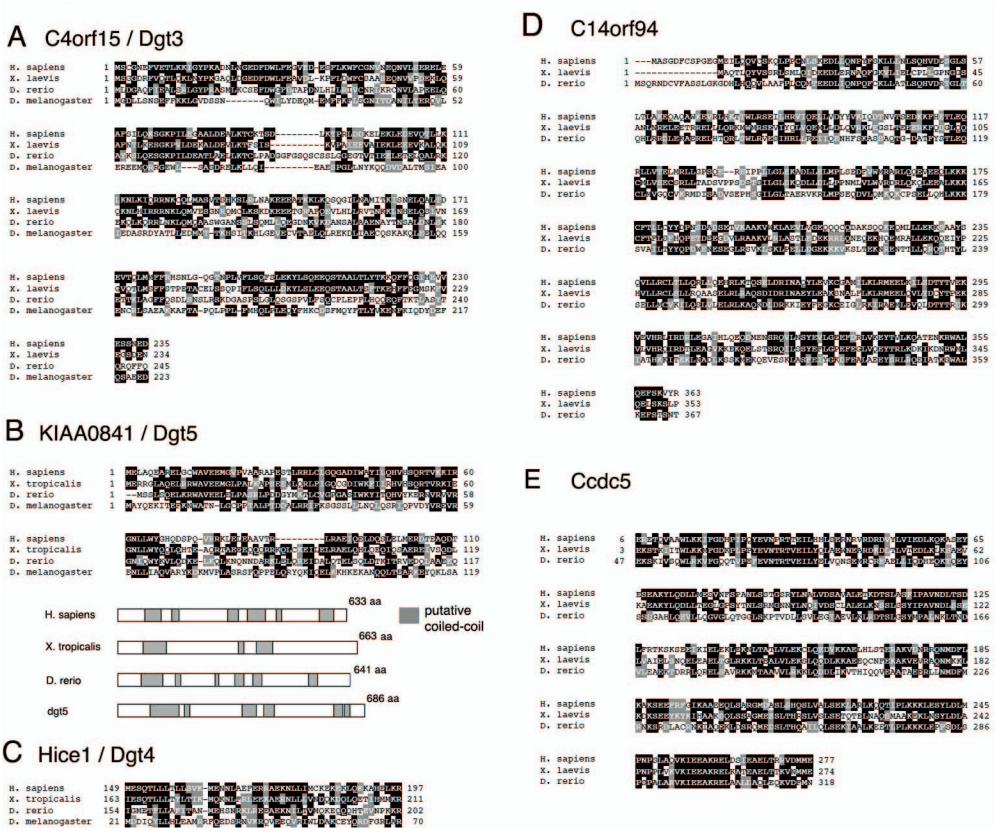
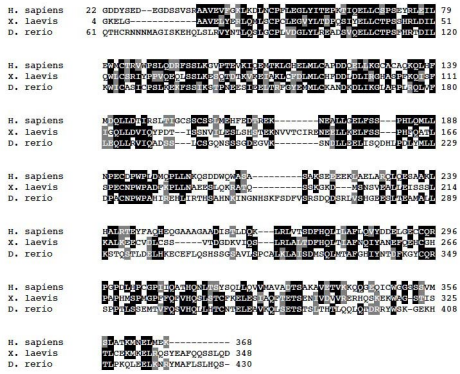


Fig. S2. Sequence alignment of Dgt3, Dgt4, Dgt5, Cep27, Ccdc5, C14orf94, and UCHL5IP. Alignment of the human augmin components with the corresponding components of *Xenopus laevis*, zebrafish (*Danio rerio*), and *Drosophila* (if present) is shown. Identical amino acids are colored black, and similar ones are gray. The predicted coiled-coil domains (COILs server, www.ch.embnet.org/software/COILS_form.html) are also illustrated for KIAA0841/Dgt5 (*B*). We performed homology search of C4orf15 (*A*), Hice1/NY-SAR48 (*C*), KIAA0841 (*B*), Ccdc5/Hei-C (*E*), Cep27 (*G*), UCHL5IP (*F*), and C14orf94 (*D*) by using BLAST and MEME/MAST, and found that C4orf15, Hice1, and KIAA0841 possess similarity to *Drosophila* Dgt3, -4, and -5, respectively. Homology between C4orf15 and Dgt3 was relatively high (21.3% identity in the N-terminal 230-aa region) and was apparent in both search methods, whereas homology of the other 2 pairs was very low and appeared only in the MEME/MAST search. However, KIAA0841 has several predicted coiled-coil domains, the locations of which are conserved in *Drosophila* Dgt5, which strongly suggests that KIAA0841 is the orthologue of Dgt5 (*B*). The similarity of Hice1 to Dgt4 is also very limited, but it interacts with hDgt6 in a yeast 2-hybrid assay (Fig. 2*D*), reminiscent of the *Drosophila* Dgt4–Dgt6 2-hybrid interaction [Giot L, et al. (2003) A protein interaction map of *Drosophila melanogaster*. *Science* 302:1727–1736]. These data suggest that Hice1 is the Dgt4 orthologue. Thus far, *Drosophila* counterparts of Ccdc5, Cep27, C14orf94, and UCHL5IP have not been identified, although all these proteins are highly conserved in vertebrates. These proteins might be functional homologues of *Drosophila* Dgt2, -7, -8, and -9, whose vertebrate counterparts have also not been identified.

F UCHL5IP



G Cep27

Fig. S2 continued.

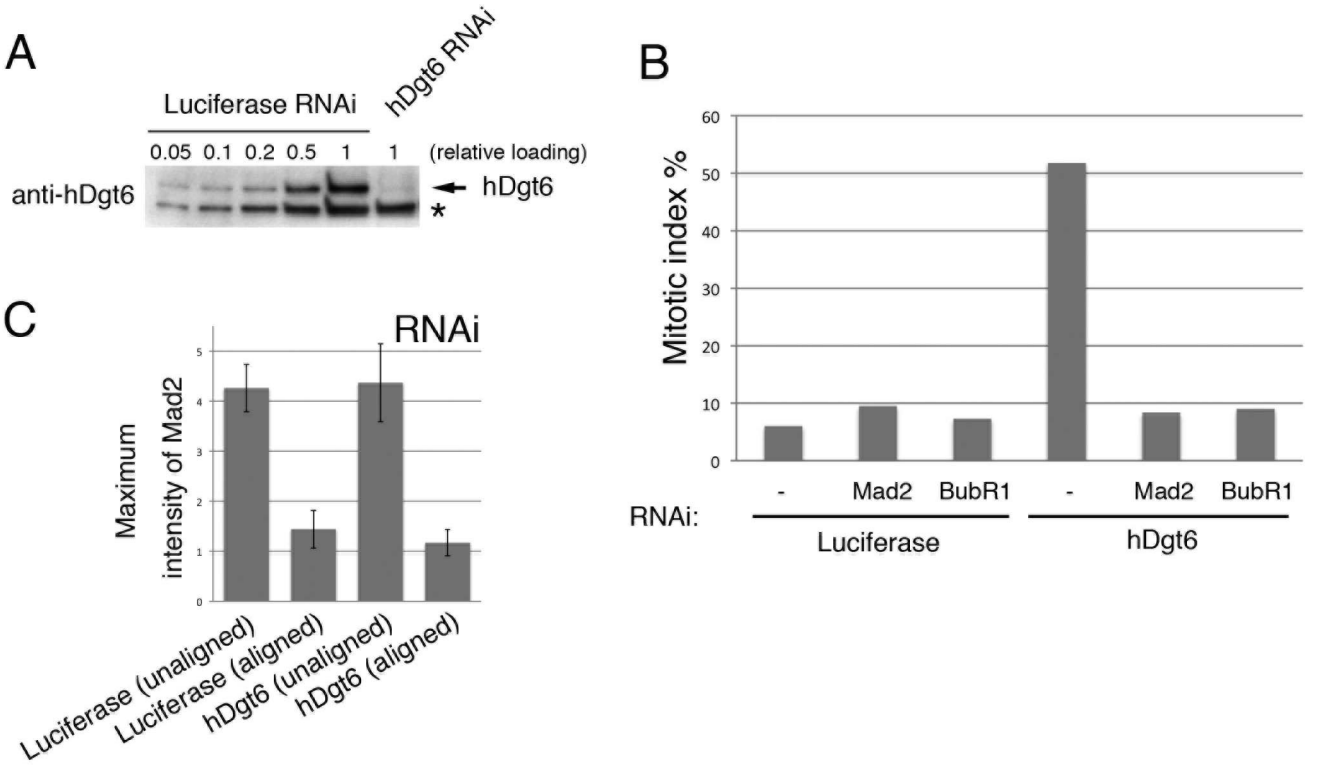


Fig. S3. Severe mitotic arrest in augmin-depleted cells. (*A*) More than 95% of the hDgt6 protein was depleted after RNAi in the optimized experimental condition. The asterisk indicates cross-reaction of anti-hDgt6 antibody to another protein, which serves as a loading control. (*B*) Mad2 and BubR1-dependent accumulation of mitotic cells after hDgt6 RNAi. Simultaneous knockdowns of Mad2 or BubR1 restored the mitotic index, indicating the mitotic delay is spindle checkpoint dependent. (*C*) The brightest kinetochore Mad2 signal in each cell was comparable between control Luciferase or hDgt6 RNAi-treated samples ($\pm$ SEM, $n \geq 3$ each).

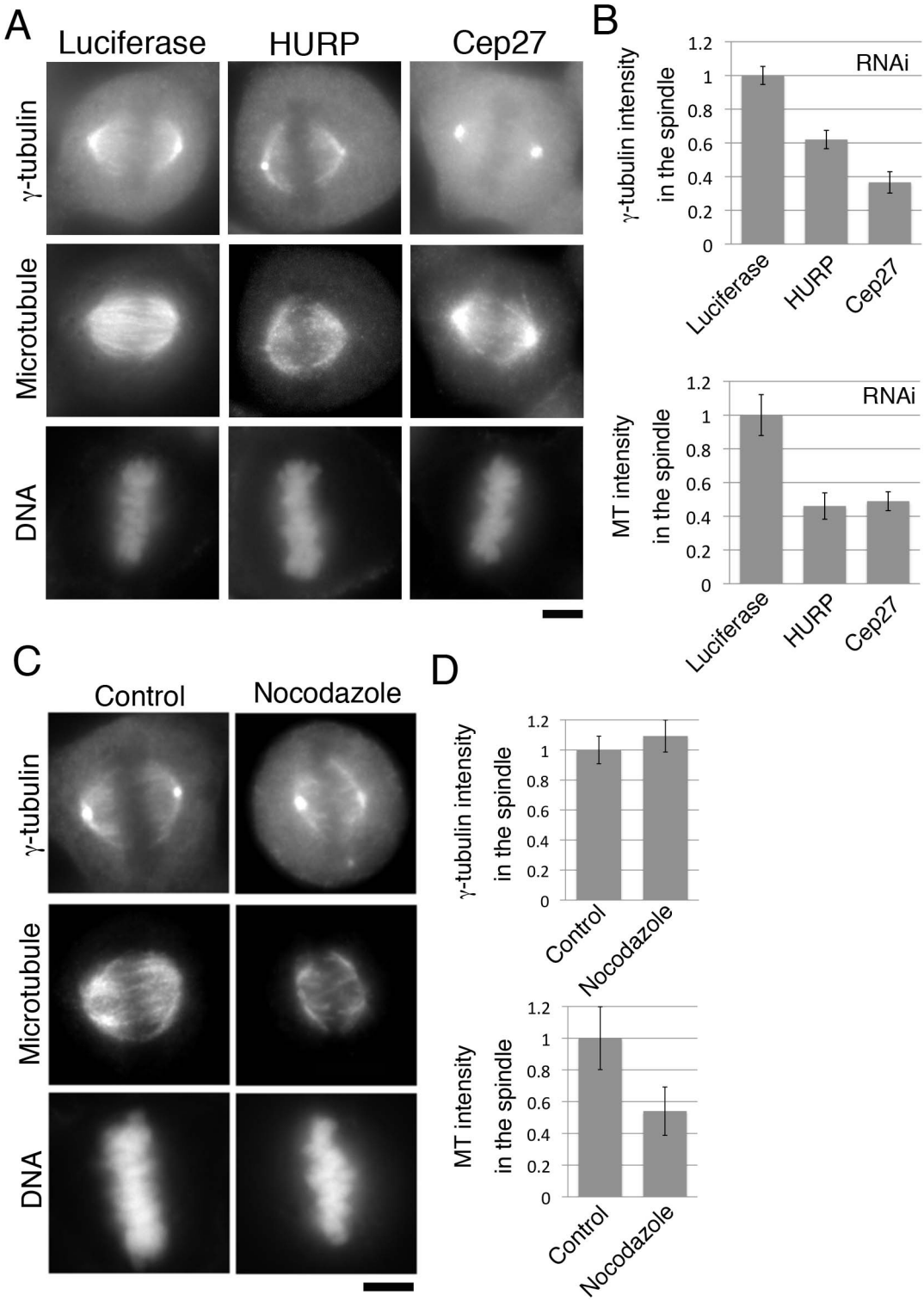


Fig. S4. Reduction of spindle MTs by HURP RNAi or nocodazole does not result in the loss of spindle γ-tubulin. (*A*) HURP RNAi resulted in reduction of spindle MTs in an extent similar to Cep27 depletion, whereas reduction of γ-tubulin was less severe in HURP-depleted cells than in Cep27-depleted cells. (*B*) Normalized intensity of spindle γ-tubulin or MTs. The intensity (±SEM) of spindle γ-tubulin was 1 ± 0.05 ($n = 6$) in control cells, 0.61 ± 0.05 ($n = 9$) in HURP-depleted cells, and 0.36 ± 0.06 ($n = 6$) in Cep27-depleted cells [significantly ($P < 0.01$) lower than that in HURP-depleted cells]. The spindle MT intensity was 1 ± 0.12 ($n = 6$) in control cells, 0.46 ± 0.07 ($n = 9$) in HURP-depleted cells, and 0.48 ± 0.05 ($n = 6$) in Cep27-depleted cells. (*C*) Spindle MTs but not γ-tubulin within the spindle were reduced in cells treated with 20 ng/mL nocodazole for 5 h. (*D*) Normalized signal intensity of spindle γ-tubulin or MTs. The intensity of γ-tubulin was 1 ± 0.09 ($n = 5$) in untreated cells and 1.09 ± 0.10 ($n = 5$) in nocodazole-treated cells. The intensity of spindle MT was 1 ± 0.19 ($n = 5$) in untreated cells and 0.54 ± 0.15 ($n = 5$) in nocodazole-treated cells. (Scale bars, 5 μm.)

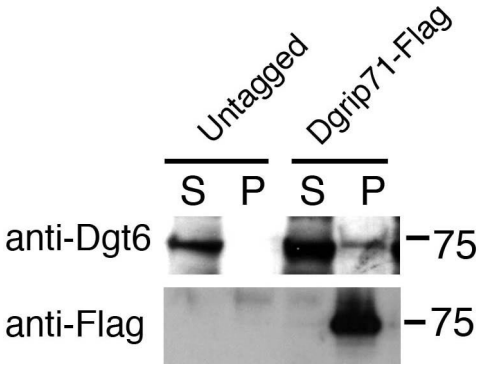


Fig. S5. Coimmunoprecipitation of Dgrip71-Flag and Dgt6 in S2 cell extracts. The supernatant (S) and precipitated (*P*: 40× volume of S) fractions after immunoprecipitation with anti-Flag antibody were immunoblotted.

Table S1. List of proteins found in the Dgt2-HA immunoprecipitates

Gene name	Protein molecular mass, AMU	No. of peptides matched	The dgt phenotype
CG8828/Dgt5	77,963.80	54	Yes
CG11881/Dgt6	72,811.00	39	Yes
CG16969/Dgt2	25,827.70	34	Yes
CG3221/Dgt3	62,560.90	25	Yes
CG13914	15,651.00	15	Yes
CG2050/modulo	60,378.30	14	No
CG4865/Dgt4	21,407.50	12	Yes
CG2213	28,444.30	12	Yes
CG11949/coracle	184,138.50	11	No
CG13879	17,507.60	10	Yes
CG7752/Z4	105,060.50	9	No
CG8200/flotillin	47,116.80	8	No

Proteins immunoprecipitated with Dgt2-HA were analyzed by LC/MS/MS. All the hits with >5 peptides uniquely detected in the Dgt2-HA sample are listed. Table columns: name of gene encoding protein, protein molecular mass from amino acid composition (atomic mass units), number of matched peptides, and the appearance of the dgt phenotype in reevaluation of the RNAi screen database [Goshima G, et al. (2007) Genes required for mitotic spindle assembly in *Drosophila* S2 cells. *Science* 316:417–421].

Table S2. Primer sequences used to generate dsRNAs for RNAi

Gene	Forward	Reverse
KIAA0841	taatacgactcactatagggagtggggatgggcttggcgac	taatacgactcactatagggacaggcgggtcttgctggcc
Dgt7	taatacgactcactatagggtaagcacctctacgaacatctcc	taatacgactcactatagggctacaacgtaccaactcaacgc
Dgt8	taatacgactcactataggggcacttcgatgaccagttaaagc	taatacgactcactatagggccaacataactacgtccctagtcg
Dgt8	taatacgactcactatagggtgtcatctccagtaaaaatgcg	taatacgactcactatagggaccctctgattgtgtagtgttcc
Dgt9	taatacgactcactatagggctggctaagatagacgagataatgg	taatacgactcactatagggttcctgtgagttcttcatctgg
Dgt9	taatacgactcactatagggacgagtcatttagtactggcgg	taatacgactcactatagggggtgcttacctatacgcttctcc

The first 20 nucleotides correspond to the T7 sequences. For Dgt8 and Dgt9, two nonoverlapping dsRNAs were used to eliminate the possible off-target effect for the phenotype.

Table S3. List of proteins found in the GFP-hDgt6 immunoprecipitates

Protein name	Protein molecular mass, AMU	No. of peptides matched	*D. melanogaster* orthologue
hDgt6/FAM29A	108,606.00	27	Dgt6
KIAA0841	71,665.00	26	Dgt5
UCHL5IP	47,244.40	25	?
C4orf15	69,634.90	22	Dgt3
RBM7	30,485.70	22	CG11454
NY-SAR-48/Hice1	44,840.90	21	Dgt4
CCDC5	31,846.50	18	?
Cep27	26,916.10	15	?
C14orf94	42,382.60	14	?
CSDA	31,928.60	13	Yps

Proteins immunoprecipitated with GFP-hDgt6 were analyzed by LC/MS/MS. All the hits with >5 peptides uniquely detected in the GFP-hDgt6 sample are listed. Table columns: name of gene encoding protein, protein molecular mass from amino acid composition (atomic mass units), number of matched peptides, and *Drosophila melanogaster* orthologues.

Table S4. Sequences of siRNA used for RNAi in HeLa cells

Gene	Sense	Antisense
C14orf94	AGCGGCUGCUUGUAACUGAtt	UCAGUUACAAGCAGCCGCUgt
C4orf15	GGACAUAUAUCGUCAACUUtt	AAGUUGACGAUAUAUGUCCcg
Ccdc5	GCGAGAACUAGAUAGCAUUtt	AAUGCUAUCUAGUUCUCGCtt
Cep27	CUUUCAUUGAGAGAUUAGAAA	UCUAAUCUCUCAAUGAAAGUC
Hice1	GAACAAUCUUGCUGAGUUUtt	AAACUCAGCAAGAUUGUUCtt
UCHL5IP	CGCUUAGAACGGAGUACUUUG	AAGUACUCCGUUCUAAGCGCG
hDgt6	CAGUUAAGCAGGUACGAAAtt	CGUACGCGGAAUACUUCGAtt

The 3′ DNA overhang is shown in lowercase.